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Center for Microbial Biotechnology
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Effect of kinetic parameters on heterologous protein production: a sysbio approach

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Summary: To improve the expression and secretion of heterologous proteins in the filamentous gram-positive bacteria, *Streptomyces lividans*, bioprocessing strategies were developed. A mathematical model was constructed to study the effects of the process kinetic parameters on the production of the model-protein RFP. The model describes the dynamics of glucose consumption and formation of biomass in addition to RFP production. The model was also extended to describe a two substrate condition.

Background

✓ Due to it´s ability to secrete proteins and low protease content, Streptomyces have been considered as an alternative host organism for producing recombinant proteins.

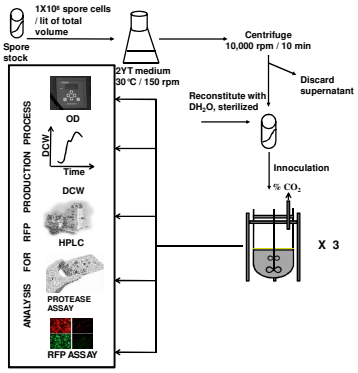
✓ In addition to genetic strategies, bioprocessing strategies are also important for improved production of protein secretion from Streptomyces.

✓ In this work, a mathematical kinetic model of the system for RFP production was developed. Laboratory data from batch fermentations involving growth and protein production by *S. lividans* on a single substrate, glucose was used to estimate parameters in the model. The model was then also extended to describe growth and production on double substrates, glucose and glutamate.

Objective

To develop a kinetic model for the prediction of protein production from *S. lividans*.

Schematic representation of process



Protease assay: A2382 Sigma kit is used for this assay, Fluorescense assay: 530/25 excitation and 600/25 emission, measured from biotek-plate reader.

The Model

✓ Growth Rate

$$\mu = \mu^m * \frac{S}{K_m+S} \quad \text{..... (1)}$$

μ: specific growth rate, (h^{-1}),

S: Substrate concentration, (g/L),

μ^m: max. specific growth rate, (h^{-1}),

K_m: Monod constant (g/L) .

✓ Temperature Effect

$$\frac{dX}{dt} = \mu * X - K_d * X \quad \text{..... (2)}$$

K_d: Specific death rate, (h^{-1})

✓ Nutrient consumption and Product formation

Substrate accumulation = Substrate Feed - Growth Formation - Product - Maint. Reqd.

$$-\frac{dS}{dt} = \frac{F*S_o}{V} - \frac{\mu*X}{Y_{SX}} - \frac{q_P*X}{Y_{SP}} - m*X \overset{def}{=} \frac{\mu*X}{Y_{SX}} - m*X \quad \text{..... (3)}$$

Y_{SX}: Biomass yield on glucose

✓ Product Formation

Product accumulation = Formation – Destruction

$$\frac{dG}{dt} = \left(\alpha_G * \frac{\mu^m*S}{K_m+S} + \beta_G\right) * X - K_{dG} * G \quad \text{..... (4)}$$

$$\frac{dG}{dt} = r_G * X - K_{dG} * G \quad \text{..... (5)}$$

$$\frac{dP}{dt} = r_P * X = (\alpha_P * \mu + \beta_P) * \left(\frac{K_{SP}}{K_{SP}+S}\right) * X \quad \text{.... (6)}$$

$$\frac{dP}{dt} = r_P * X = \left(\alpha_P * \frac{\mu^m}{K_m+S} + \beta_P\right) * \left(\frac{K_{SP}}{K_{SP}+S}\right) * X \quad \text{.. (7)}$$

Where,

r_P: Specific production rate of protease,

r_G: Specific production rate of GFP,

K_{dG}: GFP degradation rate by protease,

$r_P=(\alpha_P*\mu + \beta_P)*(K_{SP}/(K_{SP} + S))$ and $r_G=(\alpha_G*\mu + \beta_G)$

Materials and Methods

S. lividans TK24 was used throughout the study.

The medium used in this study is (/L): $NaH_2PO_4.H_2O$ (1.38g), NH_4Cl (2.675g), KCl (0.7455 g), Na_2SO_4 (0.284 g), Citric acid (0.42 g), $MgCl_2.6H_2O$ (0.254 g), $CaCl_2.2H_2O$ (0.184 g), Trace metal solution (5ml), Antifoam (sigma 204, 0.2 ml), Vitamin (1ml), $Glucose.H_2O$ (10/20/30/40 g).

Results

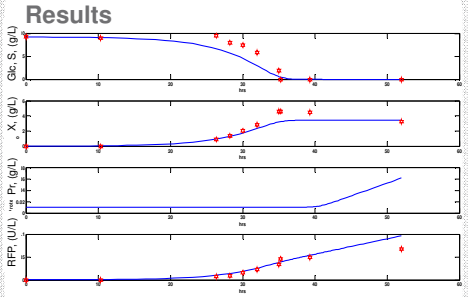


Figure 1: The single substrate kinetic model after optimization. Kinetic parameters are shown below. Lines are predictions and dots experimental data.

μ^m: 0.2, (h^{-1}), K_m: 1.35 (g/L) , Y_{SX}: 0.37. The model is validated with different initial glucose concentrations example, 20, 30 and 40 g/L.

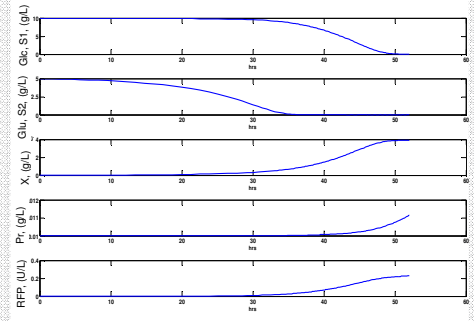


Figure 2: The double substrate kinetic model as a visualization. Kinetic parameters are: μ^{m1}: 0.2, (h^{-1}), K_m: 1.35 (g/L) , Y_{SX}: 0.37, μ^{m2}: 0.1, (h^{-1}), K_{m1}: 1. 5 (g/L) , K_{m2}: 1.5. The plan for near future is to validate this model with different initial substrate concentrations for example, Glc/Glu 20/10, 30/15 and 40/20 g/L.

Conclusions

Comparison with experimental data demonstrated that the model is able to accurately predict the protein production process kinetics. The model will be further applied to simulate different process conditions in order to gain a better understanding of the influence of different parameters on protein production.

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